Synthetic nucleic acid particle

The invention relates to a synthetic nucleic acid particle or particles, a process for its preparation and a use.

It is known that mononucleotides bind to clupeines (G. D'Auria, L. Paolillo, R. Sartorio and S. Wurzburger (1993): Structure and function of protamines: an ^1H nuclear magnetic resonance investigation of the interaction of clupeines with mononucleotides, Biochem. Biophys. Acta, 1162, 209-216).

Also known in the prior art is the preparation of complex compounds between double-stranded oligonucleotides, polycationic polymers and lipids. Concerning this, reference is made to the following publications:

A.V. Kabanov and V.A. Kabanov (1995): DNA Complexes with polycations for the Delivery of Genetic Material into Cells, Bioconjugate Chem., 6, 7-20;

Gao and L. Huang (1996): Potentiation of Cationic Liposome-Mediated Gene Delivery by Polycations, Biochemistry, 35, 1027, 1036;

L. Sorgi, S. Bhattacharya and L. Huang (1997): Protamine sulfate enhances lipid-mediated gene transfer, Gene Therapy, 4, 961-968;

Li and L. Huang (1997): In vivo gene transfer via intravenous administration of cationic lipid-protamine-DNA (LPD) complexes, Gene Therapy, 4, 891-900.

Complex compounds of this type can be used, for example, for transfection of plasmid DNA. Where protamine bound to transferrin is used as polycation,

such complex compounds are also referred to as transferrin-protamine-DNA complexes. Complexes of this type do not form condensed DNA structures or particles (Wagner, M. Zenke, M. Cotton, H. Beug and M.L. Birnstiel (1990): Transferrin-polycation conjugates as carriers for DNA uptake into cells, Proc. Natl. Acad. Sci. U.S.A., 87, 3410-3414).

The known complex compounds can be formed only from previously formed particles or existing complexes. For this it is necessary that a lipid is also present, besides a protein. It is a disadvantage that these complex compounds cannot form particulate structures from oligonucleotides. The DNA in the complex compound is bound only by surface adsorption. It is a disadvantage that it can undergo enzymatic degradation. Finally, the known complex compounds are unsuitable for producing pharmaceuticals with a depot effect.

It is an object of the invention to eliminate the disadvantages of the prior art. It is particularly intended to indicate a stable synthetic particle and a process [lacuna] its preparation which makes a high transfection efficiency possible. It is intended where possible for the synthetic particle also to be suitable for producing pharmaceuticals with a depot effect.

This object is achieved by the features of Claims 1, 11 and 24. Expedient developments are evident from the features of Claims 2 to 10, 12 to 23 and 25 to 27.

According to the invention, a synthetic particle is formed from at least one nucleic acid sequence or nucleic acid derivative sequence and one protein having a molecular weight of 3900 to 4300. Such a synthetic particle is, in particular, stable to enzymatic degradation. It makes a high transfection efficiency

possible and makes it possible to produce pharmaceuticals with a depot effect.

According to one developmental feature, the protein consists predominantly of arginine. It is advantageous for the arginine content to be more than 60% by weight. The protein may be selected from the following group: protamine, protamine base, protamine derivatives or salts, preferably protamine sulfate or protamine chloride. The aforementioned compounds advantageously have no antigenic properties.

The nucleic acid sequence, which is advantageously in single-stranded form, may be an oligonucleotide or a derivative thereof. The oligonucleotide preferably consists of at least 5 nucleotides. The derivative may be a phosphorothioate or an anionic derivative. The oligonucleotide may be, in particular, a DNA oligonucleotide. This makes it possible to use the synthetic particles for antisense therapy.

The average diameter of the particle can be in the range from 10 nm to 100 µm, depending on the purpose of use.

The particle advantageously carries a surface electric charge which may preferably be in the range from -40 mV to +40 mV. This makes it possible to increase the transfection efficiency further.

According to the process of the invention there is provision of a process for the preparation of the synthetic particles according to the invention, with the following steps:

a) preparation of an aqueous first solution containing a protein having a molecular weight in the range from 3900 to 4300,

b) addition to the first solution of a second solution containing a nucleic acid sequence or nucleic acid derivative sequence and

c) mixing of the first and second solution.

The process makes it possible to prepare the synthetic particles according to the invention in a simple manner.

According to one developmental feature, the first and the second solution are free of salts. It is possible to adjust the molar ratio of nucleic acid sequence or nucleic acid derivative sequence to protein to produce a predetermined surface charge. The proposed variant can be carried out particularly simply.

The protein expediently consists predominantly of arginine, and it can be selected from the following group: protamine, protamine base, protamine derivatives or salts, preferably protamine sulfate or protamine chloride. Protamine, protamine base or protamine derivatives in particular can be obtained from salmon sperm. Easy and low-cost availability is thus ensured.

The nucleic acid sequence, which is advantageously in single-stranded form, may be an oligonucleotide or a derivative thereof. The oligonucleotide preferably consists of at least 5 nucleotides. The derivative may be a phosphorothioate or an anionic derivative. The diameter of the particle may be in the range from 10 nm to 100 μm, depending on the purpose of use. It may carry a surface electric charge which is expediently in the range from -40 mV to +40 mV.

According to another achievement of the object there is provision of the use of a protein having a molecular

weight in the range from 3900 to 4300 for the preparation of a synthetic particle containing at least one nucleic acid sequence or nucleic acid derivative sequence.

The protein advantageously consisting predominantly of arginine can be selected from the following group: protamine, protamine base, protamine derivative or salts, preferably protamine sulfate or protamine chloride. The nucleic acid sequence which is advantageously in single-stranded form may be an oligonucleotide preferably consisting of at least 5 nucleotides, or a derivative thereof. The derivative may be a phosphorothioate or an anionic derivative. The oligonucleotide is expediently a DNA oligonucleotide.

The synthetic particle according to the invention is advantageously formed exclusively from the nucleic acid or the nucleic acid derivative and the protein having the molecular weight in the range from 3900 to 4300. The molecular weight of the protein in a particularly advantageous embodiment is between 4000 and 4250.

Exemplary embodiments of the invention are explained in detail below by means of the drawing and by means of examples. In the drawings,

Fig. 1a shows a scanning electron micrograph of synthetic particles with a negative surface charge,

Fig. 1b shows a scanning electron micrograph of synthetic particles with a positive surface charge,

Fig. 2 shows the dependence of the particle size on the incubation time,

Fig. 3 shows the dependence of the surface charge on the protamine/oligonucleotide ratio,

Fig. 4a shows a confocal laser scanning micrograph of a first vero cell.

Fig. 4b shows a confocal laser scanning micrograph of a second vero cell.

Fig. 5 shows the dependence of the UV absorption at 260 nm on the retention time for particles differing in protamine/oligonucleotide composition.

Examples:

1. Preparation of oligonucleotide particle with negative surface charge

500 µl of a protamine solution (50 µg/ml) in double-distilled water are spontaneously added in an Eppendorf cap at room temperature to 500 µl of a likewise salt-free oligonucleotide solution (100 µg/ml). The oligonucleotides preferably present in the solution are single-stranded DNA oligonucleotides. The solution is then vigorously mixed for 1 minute with a high-speed stirrer. Particle formation starts spontaneously and is complete after half an hour. The ratio by weight between the protamine molecule employed and the oligonucleotide is about 0.75 to 1. The ratio by weight between protamine and the oligonucleotide for particle formation is about 1:2.5.

2. Preparation of oligonucleotide particle with positive surface charge

Based on the process described in Example 1 process [sic], 500 µl of a protamine solution (250 µg/ml) in

double-distilled water are added spontaneously in an Eppendorf cap at room temperature to 500 µl of a likewise salt-free oligonucleotide solution (100 µg/ml). The molar ratio between protamine and the oligonucleotide is about 3:1.

Fig. 1a shows a scanning electron micrograph of a synthetic particle with negative surface charge. The ratio by weight between protamine and oligonucleotide in this case was 1:2. Fig. 1b shows a synthetic particle with positive surface charge. The ratio by weight between protamine and oligonucleotide in this case was 2.5:1.

Fig. 2 shows the dependence of the incubation time on the protamine/oligonucleotide ratio by weight. The particle size increases with increasing incubation time. It is thus possible to adjust any desired particle sizes.

Fig. 3 shows the dependence of the zeta potential on the protamine/oligonucleotide ratio by weight. As the protamine content increases, the zeta potential is shifted to positive values.

Figs. 4a and b show comparatively the uptake of oligonucleotides by means of synthetic particles (Fig. 4a) in vero cells. Fig. 4b shows a control incubation of dissolved oligonucleotides. The oligonucleotide concentration is 5 µg/ml with an incubation time of four hours at 37°C and 5% CO_2. It is evident that the uptake of oligonucleotides in cells is increased on use of the synthetic particles according to the invention.

Fig. 5 shows the stability of the particles according to the invention to enzymatic degradation by endonucleases. The UV absorption at 260 nm is plotted against the retention time for various

protamine/oligonucleotide ratios. The results show that the particle according to the invention ensures virtually quantitative protection from enzymatic degradation.